Green Synthesis of New Neurotransmitters based on Indole Moiety

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted By

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To whome it may concern

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Priyansha is very sincere, punctual, disciplined and obedient. I have always noticed a great deal of determination in Priyansha and she is an enthusiastic student who always strives for new knowledge. I wish her every success in life.

Sincerely,

Piyali Sarkar

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Supervisor's Approval

This is to certify that the thesis titled **'Green Synthesis of New Neurotransmitters based on Indole Moiety'** submitted by **Priyansha Moulik**, a postgraduate student of **Sister Nivedita University, Kolkata**, has successfully completed her dissertation work under our supervision. This report has been reviewed and approved for submission in partial fulfillment of the requirements for the degree Master of Science.

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DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Green Synthesis of New Neurotransmitters based on Indole Moiety" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from 13/01/2025 to 20/05/2025 under the supervision of Dr. Piyali Sarkar, Department "Institute of Health Sciences". I acknowledge that I have read and understood the UGC regulations, 2018 (Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions).

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Priyansha Moulik
05.06.25

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TO WHOM IT MAY CONCERN

This is to certify that the project report titled "Green Synthesis of New Neurotransmitters based on Indole Moiety", submitted by Ms. Priyansha Moulik (Registration No. 230020150274, Enrolment No. 2322210002015) to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by her for the partial fulfillment of the requirements for the Master of Science degree.

Date: 03.06.2025

Place: SISTER NIVEDITA UNIVERSITY

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ABBREVIATIONS

S/N	ABBREVIATION	MEANING
1	NTs	Neurotransmitters
2	BBB	Blood Brain Barrier
3	CNS	Central Nervous System
4	5-HT	5-hydroxytryptamine
5	RB	Round Bottom Flask
6	NMR	Nuclear Magnetic Resonance
7	DMSO	Dimethyl Sulfoxide
8	e.g.	For Example
9	i.e.	That is
10	Etc.	Et cetera

ABSTRACT

Neurotransmitters are essential chemical messengers that enable communication between neurons and their target cells, orchestrating a wide range of physiological processes including mood regulation, cognition, muscle movement and organ function. Despite their critical roles, many naturally occurring neurotransmitters face a significant limitation, this being their inability to efficiently cross the blood brain barrier (BBB), a highly selective membrane. It protects the brain but also restricts the entry of therapeutic agents that could target CNS related diseases. This barrier poses a major challenge in treating neurological disorders such as depression, Parkinson's disease and Alzheimer's disease, where effective delivery of neurotransmitters or their analogues to the brain is crucial. As the prevalence of these conditions rises, there is an urgent need to develop new neurotransmitter molecules that not only possess enhanced pharmacological properties but are also capable of traversing the BBB to reach their targets in the central nervous system. In this context, we will throw light on indole moiety, a structural motif found in many biologically active compounds, that offers promising potential for the design of novel neurotransmitters. This thesis focuses on the green synthesis of new indole based neurotransmitter analogues, employing environmentally friendly methods to minimize hazardous waste and energy consumption. The synthesized compounds are evaluated for their ability to form synaptic networks among neuronal cells, checking their cytotoxicity and further testing in the future if it is able to cross the BBB with the aim of identifying candidates that could address multiple neurological disorders. By integrating principles of green chemistry with innovative molecular design, this research aspires to contribute to the development of safer, more effective therapeutics for complex brain diseases.

Key words: Neurotransmitters, Indole derivatives, Green synthesis, Neurodegenerative diseases, Blood Brain Barrier, Pharmacophores, Molecular Design

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1. INTRODUCTION

Neurotransmitters are specialized chemical messengers, they play a fundamental role in the functioning of the nervous system by transmitting signals between neurons and other target cells. They are really important for regulating several psychological and physiological processes, this includes our mood, movement, heartbeat and blood pressure, hormone regulation, memory, digestion etc. [1] Synapses are the specialized points where neurons communicate. Each synapse features two main parts: a postsynaptic and a presynaptic terminal. The release of NTs from the presynaptic neuron into the synaptic cleft is initiated by an electrical impulse. These NTs then bind to postsynaptic receptors, either stimulating or inhibiting subsequent signaling. [2] Based on their effects, neurotransmitters are broadly classified as excitatory, inhibitory, or modulatory. Major classes include amino acids (e.g. glutamate, GABA), monoamines (e.g. dopamine, norepinephrine, epinephrine, serotonin), peptides (e.g. endorphins), purines and others. [3],[4] Neurotransmitters hence are in control of several actions that are necessary for our survival but when our body faces an imbalance or dysfunctions in their signaling, presence or action, it implies a variety of neurological and psychiatric disorders. [1] Now among all these we shall throw light on Serotonin (5-hydroxytryptamine or 5-HT), a monoamine neurotransmitter that also acts as a hormone. Studies have shown how 5-HT has a strong influence on both peripheral and physiological activities. [5] In our human body, almost 95% of 5-HT is found in the gut (enterochromaffin cells) and the remaining 5% in the brain. [6] It is able to regulate our mood, digestion, sleep etc. Low levels can cause conditions such as depression, anxiety, migraine, and Parkinson's disease. [7] Not only this but it can also modulate other neurotransmitters' activity, this includes dopamine, glutamate and GABA. [8]

Serotonin hence is of high importance but there comes a huge problem i.e the Blood Brain Barrier (BBB). The brain is protected by the BBB is a specialized membrane that allows only certain substances to pass through due to its highly selective and semi permeable nature. It restricts the entry of toxins, pathogens and even several therapeutic agents, this includes most of the naturally occurring neurotransmitters and their analogues. [9] Because of this limitation numerous

CNS related diseases and disorders are not getting effective management and treatment since when these neurotransmitters are administered exogenously, they fail to reach the target within the brain. [10] As per recent studies it was found that neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease are characterized by progressive loss of neuronal structure and function, leading to weakening cognitive and motor deficits, they are also one of the main causes of disability and death worldwide.

[11] Current therapeutic strategies are limited by poor BBB permeability, suboptimal efficacy and undesirable side effects, underscoring the necessity for innovative approaches in drug design and delivery. [12] To tackle this problem it's the need of the hour to design and synthesize neurotransmitter analogues that not only enhances the properties of NTs but can also cross the BBB. This could revolutionize medicine and introduce effective ways to treat patients in need. In recent years, green chemistry has emerged as a transformative approach in pharmaceutical synthesis, emphasizing the use of environmentally friendly solvents, renewable resources and energy efficient processes. The application of green chemistry principles to the synthesis of indole-based neurotransmitter analogues offers the dual advantage of reducing environmental impact and generating safer, more sustainable small molecules that can mimic a neurotransmitter. One of the common things among several biologically active molecules is that they all have an indole ring, for example- 5-HT. By using green synthetic methodologies, it is possible to design and produce novel indole-based neurotransmitters with properties tailored for enhanced BBB penetration and therapeutic efficacy. [13], [14]

This thesis aims to address the urgent need for new neurotransmitter analogues capable of overcoming the limitations imposed by the BBB. The research focuses on the green synthesis of indole-based neurotransmitters, followed by their evaluation in biological systems to assess cytotoxicity, how well it helps the neuronal cells proliferate and communicate and BBB permeability and efficacy against neurodegenerative disease models for future studies. By integrating advances in green chemistry with neuropharmacological innovation, this work aspires to contribute to the development of next generation therapeutics for CNS disorders.

2. AIMS AND OBJECTIVES

The primary aim of this research is to synthesize small molecules capable of mimicking the action of neurotransmitters, serotonin, that regulates mood, sleep and appetite via interactions with 5-HT receptors. To create BBB-penetrant analogues, we propose structural modifications that balance lipophilicity (for membrane diffusion) and molecular weight (<450 Da) while preserving receptor-binding affinity, specifically by utilizing environmentally sustainable green chemistry methods. The study will focus on designing and developing pharmacophores through eco-friendly synthetic routes, employing safer solvents such as water and ensuring the recovery and reuse of solvents and catalysts to minimize environmental impact. Once synthesized, these compounds will be characterized in order to check their purity and confirming whether our target product is synthesized or not, this is carried out by techniques such as NMR (^{1}H & ^{13}C) which is used for determining the molecular weight that further confirms the molecular structure and purity of our synthesized product. To check our compound's activity it is fully purified and analysed before being evaluated for their biological activity, specifically their ability to interact with neurotransmitter receptors or pathways in biological systems. An important aspect of the research involves exploring how combining different compounds can result in molecules with enhanced and more specific neurotransmitter-like functions. Throughout the project, special emphasis will be placed on optimizing synthetic methodologies to reduce waste generation and ensure the overall sustainability of the process. Additionally, the environmental benefits of the adopted green chemistry approaches will be assessed in comparison to conventional methods, with a focus on achieving lower toxicity, improved biodegradability, and a reduced ecological footprint. Hence by integrating green chemistry with neuropharmacology, this work seeks to advance sustainable drug discovery, offering a dual benefit: potential therapeutics for serotonin linked disorders (e.g., depression, migraines) and a reduced environmental footprint compared to conventional methods.

Taking a sustainable route for the synthesis of Neurotransmitter Analogues
Keeping in mind the problems associated with NTs the current study suggests developing new BBB permeable derivatives of serotonin using greener methods.

The classical synthesis pathways for neurotransmitter analogs almost invariably utilize harmful solvents, employ toxic catalysts like heavy metals hence by following the rules of green chemistry we can contribute to a safer and cleaner synthesis method.

Our research was conducted keeping the following things in mind:

1. Our research adhered to the core principles of green chemistry that promotes sustainability and environmental responsibility.

2. Environmentally safer solvents were prioritized, water was chosen as one of the primary solvent due to its suitability for biological systems and minimal ecological impact. Chlorinated solvents were avoided as much as possible.

3. Solvents and catalysts were systematically recovered and reused throughout the experimental process, reducing resource consumption and minimizing chemical waste.

4. The design of our target compounds focused on safety, aiming to produce molecules with lower toxicity to the environment and biological systems along with improved biodegradability.

5. Experimental methodologies were optimized to generate significantly less waste, aligning with the goals of waste prevention and resource efficiency in green chemistry.

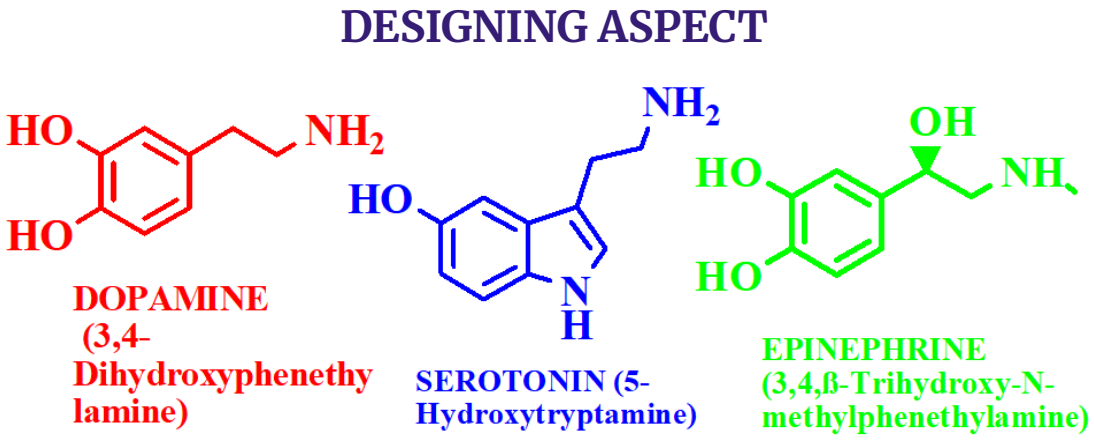


Figure 1: Amine based Neurotransmitters

Designing of our target pharmacophore starts with understanding the structural features of a neurotransmitter that provides it with the ability to show biological activity. Figure 1 highlights amine based NTs. We see how they are structurally similar to each other. Other NTs such as melatonin and tryptamine contain an indole ring and amine group. Now, serotonin can not cross the BBB but it's precursors i.e tryptamine and tryptophan can. Our aim here is to create new small molecules that can not only mimic the action of NTs (Serotonin) but also cross the BBB for the treatment of various mental health disorders. In this study our strategy is to combine an indole derivative with aniline and a phenolic compound.

REACTION 1

- **1*H*-Indole-2,3-dione + N,N-Dimethylaniline**

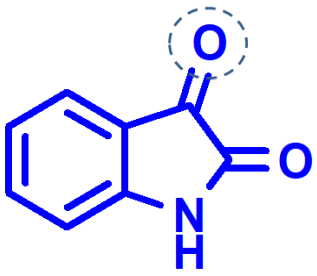


Figure 2: Active site of 1*H*-Indole-2,3-dione

For the first reaction we have chosen 1H-Indole-2,3-dione and N,N-Dimethylaniline. The active sites here being the carbonyl group at position 3 of 1H-Indole-2,3-dione and the amine group of N,N-dimethylaniline. Their interaction is expected to yield a stable product that modifies the indole structure, potentially enhancing its ability to cross the BBB.

A coupling reaction will link the indole and benzylamine scaffolds; we anticipate the yielding hybrid molecule will be stable with enhanced abilities. This product retains the below mentioned properties.

- An indole ring. Serotonin's activity critically depends on it. [15]

- The amine group. It's ability to be protonated facilitates BBB transport. [16]
- The -OH group. Solubility and hydrogen bonding capacity are enhanced by the phenolic -OH group [17]

REACTION 2

- **5-Bromoindole + 4-Hydroxybenzylamine**

For the second reaction we have chosen 5-Bromoindole and 4-Hydroxybenzylamine. 5-Bromoindole has a reactive bromide substituent (-Br) at position 5 and an aromatic indole core. 4-Hydroxybenzylamine contains a phenolic -OH group and a primary amine ($-NH_2$) on a benzyl scaffold.

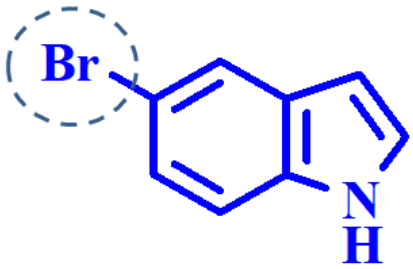


Figure 3: Active site of 5-Bromoindole

3. MATERIALS AND METHODS

3.1 MATERIALS REQUIRED

The materials required include 1*H*-Indole-2,3-dione, N,N-Dimethylaniline, 5-bromoindole, 4-hydroxybenzylamine, copper nanoparticles, and sodium sulfate anhydrous as the primary chemical reagents. Essential solvents and purification agents consist of distilled water and a range of organic solvents such

as ethyl acetate, hexane, methanol, chloroform, dichloromethane (DCM) and acetone.

The necessary laboratory equipment comprises a condenser, oil bath, rheostat, magnetic bead, magnetic stirrer, Tullu pump, Teflon tape and a rotary evaporator.

- **REACTION 1:**

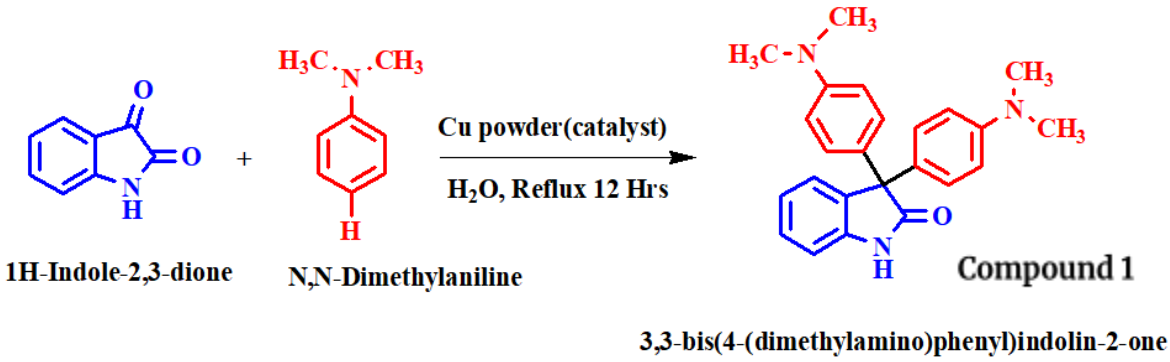


Figure 4: Reaction 1

3.2 PROCEDURE OF REACTION 1

To begin the synthesis, 1*H*-Indole-2,3-dione (~294.26 mg, 2 mmol) and N,N-Dimethylaniline (~125 µL, 4 mmol) were carefully weighed and pipetted out respectfully and then transferred to a 25mL clean and dry round bottom flask, next an approximate quantity of distilled water (~ 5ml) and catalytic amount of copper powder, a green catalyst were mixed along with the starting compounds. A reflux condenser is used for this experiment. The flask is connected to the condenser and adjusted well. This helps control the reaction environment, and the solvent does not evaporate. The entire setup is placed in a hot silicone oil bath, and the temperature is gradually raised and maintained at approximately 120°C. The reaction mixture is heated under these conditions for three days, with each day involving about 8 to 10 hours of continuous heating. This extended heating period ensures complete interaction between 1*H*-Indole-2,3-dione and N,N-Dimethylaniline, maximizing the yield and purity of the product. Throughout the process, the use of copper nanoparticles as a catalyst and water

as a solvent exemplifies green chemistry principles, reducing environmental impact and enhancing sustainability. After the reaction period, the mixture is allowed to cool before proceeding to product isolation and purification.

After the reaction is completed, the RB is carefully raised above the oil bath and positioned so any adhering oil can drip back into the bath. Once the flask has cooled and excess oil has drained off, it is detached from the setup for the next stage of processing. Next, to isolate the product, the first step begins with removing water from the reaction mixture. This is achieved using a rotary evaporator, which gently and efficiently removes solvents by rotating the flask under reduced pressure while it is partially submerged in a heated water bath. Once the water is fully evaporated, the resulting residue containing the crude product and copper catalyst is treated with a series of organic solvents such as methanol, ethyl acetate, dichloromethane (DCM), etc. These solvents are added as per the requirement that allows us to extract our crude as much as possible. The flask is gently swirled and the inner surface is scratched with a spatula to dislodge any solid product adhering to the walls, ensuring maximum recovery.

Next, the mixture sits undisturbed, allowing the denser copper nanoparticles and any insoluble material to settle. The supernatant (which holds the crude product dissolved in organic solvent) is carefully decanted into a clean china dish. Silica gel powder is then introduced and mixed well. This silica gel functions as an adsorbent, effectively capturing the product to aid in its later purification.

The mixture of silica and product is left to air dry in a china dish, allowing the solvent to evaporate, this leaves behind the crude product adsorbed onto silica. This process is repeated multiple times: fresh solvent is added to the original flask, more product is extracted and transferred to the china dish, and additional silica gel is used as needed. This iterative extraction ensures that as much product as possible is recovered from the reaction mixture. This systematic approach-combining careful solvent removal, efficient extraction, sedimentation, and adsorption-maximizes product yield and purity, setting the stage for subsequent purification steps such as column chromatography.

3.3 PURIFICATION OF CRUDE PRODUCT

The purification of the crude product is accomplished using column chromatography, a technique that is used to separate compounds based on their

differential affinities for a stationary phase (silica gel) and a mobile phase (organic solvents).

- **Column Preparation**

A clean, dry glass chromatography column was taken and prepared by cotton plugging it at the base. For the stationary phase silica gel was chosen, using our mobile phase solvent i.e Hexane we prepared a slurry that was evenly wetted and free from air bubbles. The slurry was hence poured and the solvent was allowed to drain until it just reached the surface of the silica, making sure the column never dries. The length was decided upon keeping in mind that we wanted a longer column for better separation.

- **Sample Loading**

Our crude was in dry state since we already adsorbed it in a small amount of silica gel. For loading, the powder was transferred onto the top of the silica bed, making sure that it's even. After this it was cotton plugged just above the sample bed and a small amount of hexane was poured to settle the sample and begin the elution process.

- **Elution and Fraction Collection**

Once the column was prepared we began elution with pure hexane as the mobile phase, gradually increasing the polarity by introducing Ethyl acetate in incremental concentrations. We started with 2% Ethyl acetate in Hexane, creating this gradual gradient allows efficient separation of compounds based on their polarity. At lower polarity non polar compounds elute first, polar compounds on the other hand retain for longer, only eluting out when the polarity is increased.

- **Isolation of products**

To isolate the desired compound after column chromatography, we evaporate the solvents from the collected fractions using a rotary evaporator. This process yields a solid residue within the round-bottom flask, which we must handle with care to prevent any loss or contamination. An added benefit is that the solvent collected by the evaporator can be reused for the column, promoting efficiency.

3.4 MONITORING REACTION PROGRESS

Thin Layer Chromatography (TLC) is performed to check and keep track of our reaction's progress. This technique is used to identify which fraction contains the target product. For this, on a TLC plate a small dot of sample taken from our elution alongside the starting material is taken around 1 CM above the bottom on the plate. In a TLC chamber this plate was dipped in an appropriate solvent (ex- 25% Ethyl acetate-Hexane) and left undisturbed. The plate is then visualized under UV, this is where we can monitor the movement of the compound on the plate.

3.5 CRYSTALLIZATION OF THE SYNTHESIZED PRODUCT

To turn our product into crystals we dissolve the compound formed with solvents like ethyl acetate and methanol. Once dissolved the solution is transferred to a clean and dry culture tube or conical flask and left undisturbed in a cool, dark place for several days. The resulting crystals are typically of high purity, making them ideal for subsequent cell based assays.

- **REACTION 2**

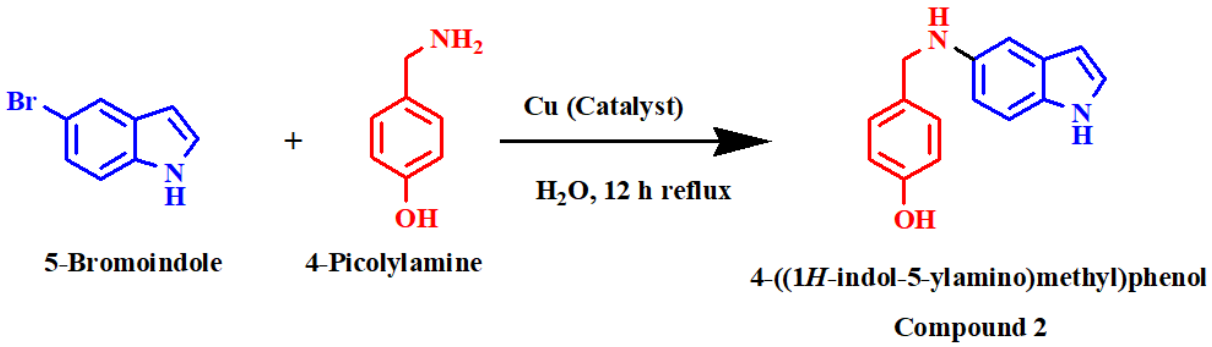


Figure 5: Reaction 2

3.6 PROCEDURE OF REACTION 2

To begin the synthesis, 5-Bromoindole (294 mg, 1.5 mmol) and 4-Hydroxybenzylamine (369 mg, 3mmol) were carefully weighed and transferred to a 25mL clean and dry RB, next we add an approximate amount of distilled water (appx~ 5ml) and a catalytic amount of copper powder were added and

mixed along with the starting compounds, then a reflux condenser is taken and attached to the flask. It is secured well with teflon tape. This setup prevents solvent evaporation and ensures a stable reaction environment. The entire setup is placed in a hot silicone oil bath, and the temperature is gradually raised and maintained at approximately 120°C. The reaction mixture is heated under these conditions for three days, with each day involving about 8 to 10 hours of continuous heating. Our yield is maximised by heating the starting materials for an extended period, this ensures complete interaction.

Rest of the steps from here are the same as reaction 1.

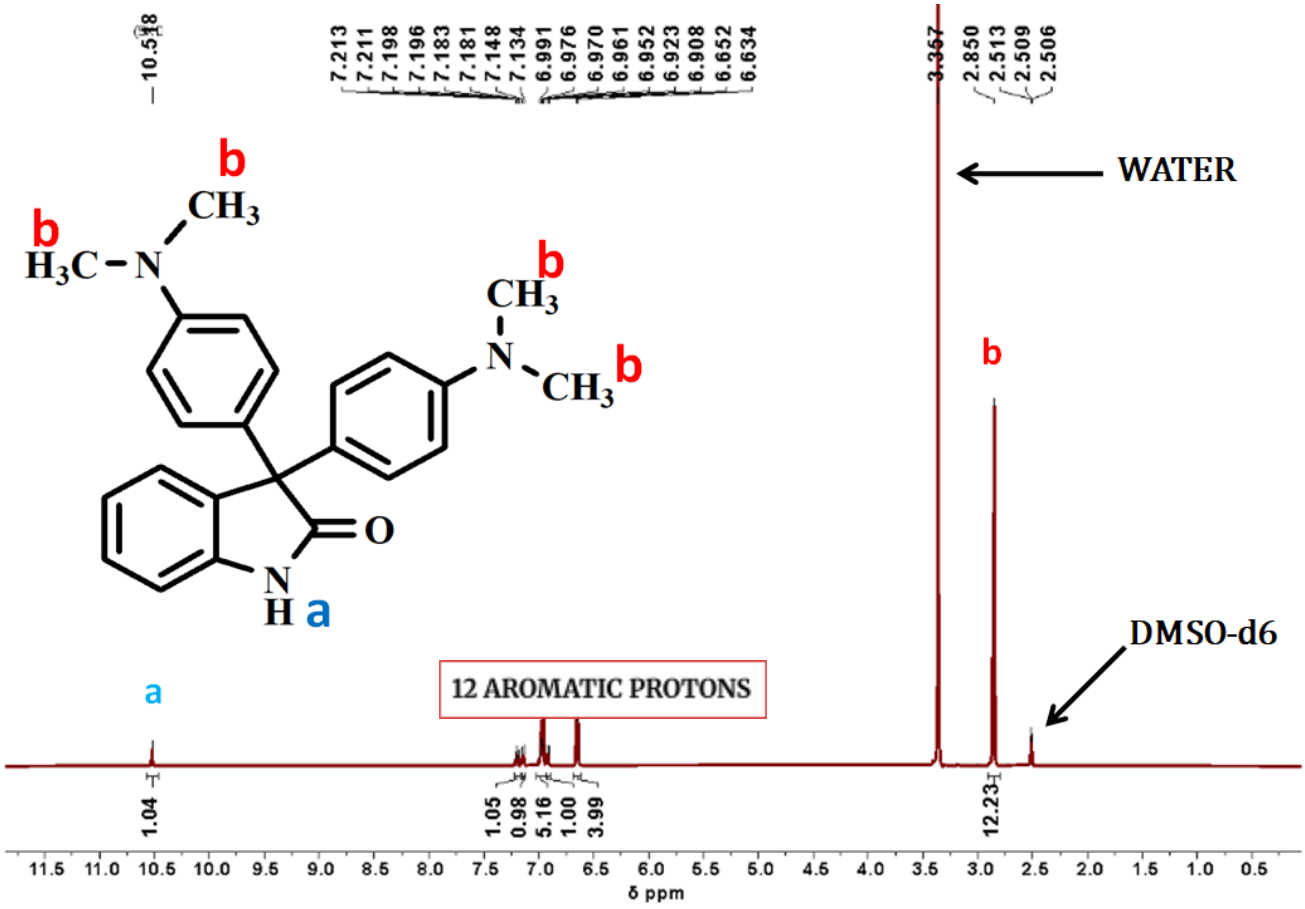
4. RESULTS AND DISCUSSION

We set aside a small sample of the dried product to be analyzed using NMR spectroscopy. For this, the sample is dissolved in an appropriate deuterated solvent (such as $CDCl_3$ or DMSO-d_6), transferred to a clean NMR tube, and prepared according to standard guidelines to ensure accurate and interpretable spectra.

4.1 NMR ANALYSIS

COMPOUND 1

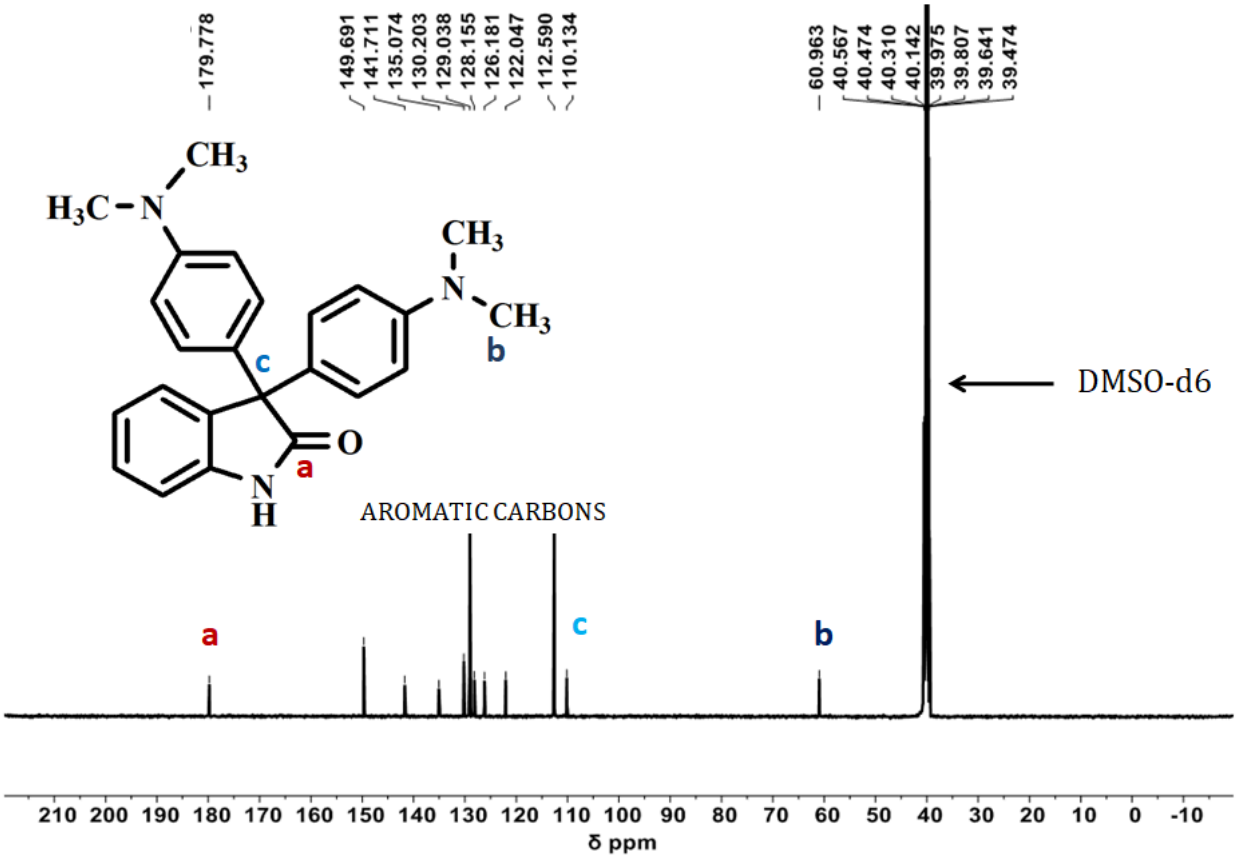
4.1.1 Characterization of Compound 1 using ^{1}H-NMR. (600MHz)



The above ^{1}H NMR spectrum indicates that:

- On the graph, b represents H from the N-CH_3 groups. In total there are 12 H protons. Peaks appeared at δ2.51 ppm. This is the representation of Aliphatic protons.
- A singlet at δ 10.51 ppm represents 1H associated with -NH. This represents the Acidic proton.
- The region between δ6.63 to δ7.21 ppm the Aromatic protons.

4.1.2 Characterization of Compound 1 using ^{13}C-NMR. (150MHz)

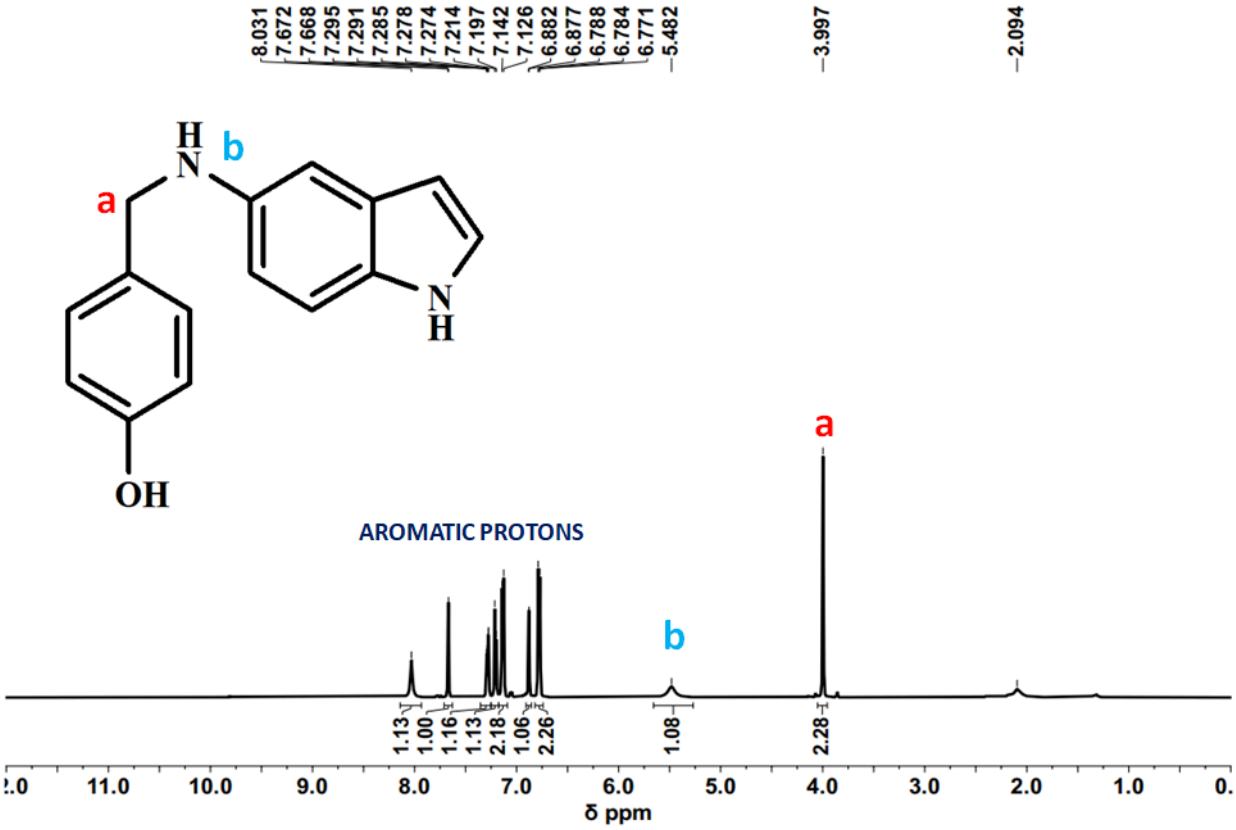


The above ^{13}C NMR spectrum indicates that:

- At δ 60.96 ppm the peak represents a methyl group attached to a Nitrogen atom (N-CH_3), denoted as b.
- From δ 112.59 to δ 149.69 ppm the peaks represent Aromatic carbons.
- The singlet at δ 179.77 ppm, represents a carbonyl carbon (C=O), denoted as a
- Here 'c' is a quaternary carbon.

COMPOUND 2

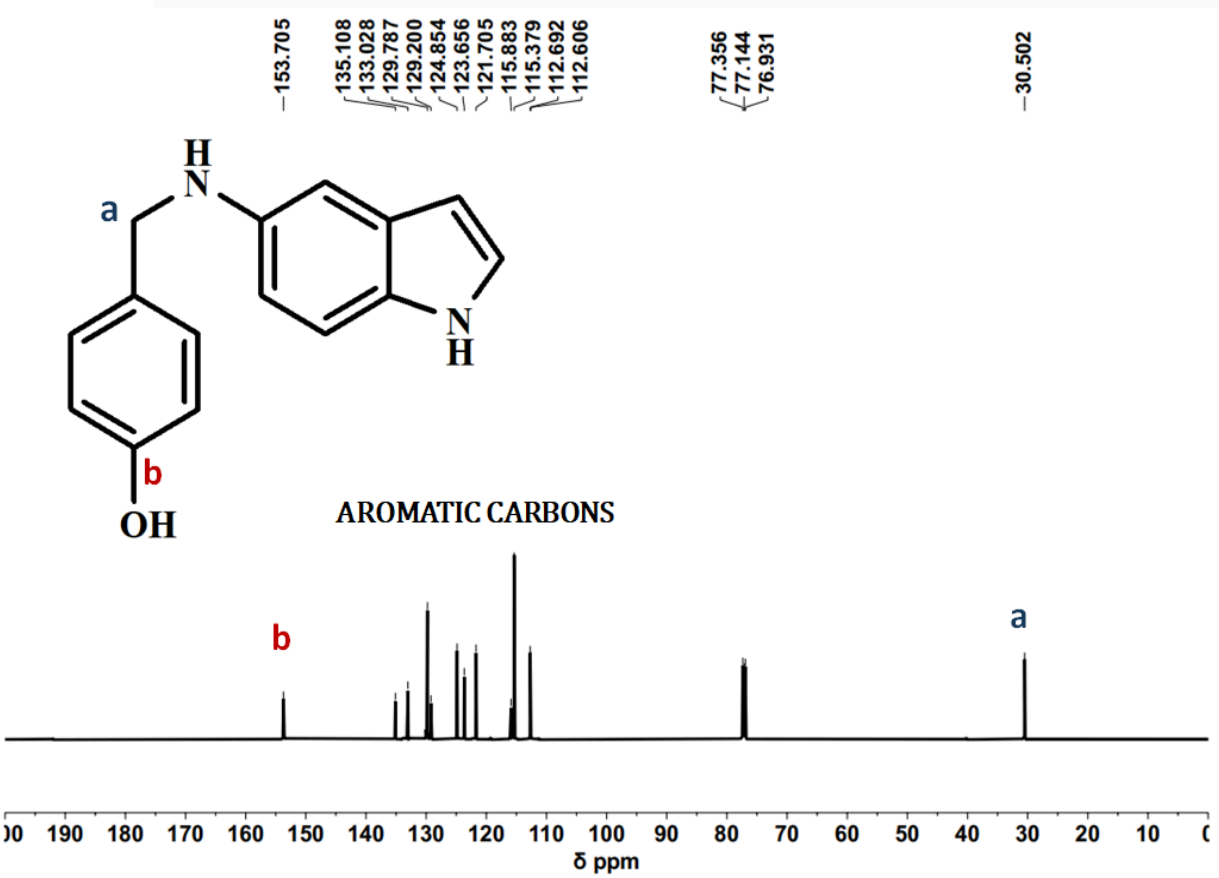
4.1.3 Characterization of Compound 2 using ^{1}H-NMR. (600MHz)



The above ^{1}H NMR spectrum indicates that:

- A signal at δ 3.99 ppm is representing an Aliphatic proton (-CH_2)
- Aromatic protons fall between the range of δ 6.77 ppm to δ 8.03 ppm in this graph. .
- At δ 5.48 ppm, the peak represents an aromatic proton. It is assigned to a carbon that is attached with an electronegative element like nitrogen.

Characterization of Compound 2 using ^{13}C-NMR. (150MHz)



The above ^{13}C NMR spectrum indicates that:

- Between δ 112.6 ppm to δ 135.1 ppm we can observe the Aromatic Carbons present in our compound.
- The Aliphatic carbon gives the signal at δ 30.5 ppm. Denoted as 'a'
- At the signal observed at δ 153.7 ppm there is an aromatic carbon. This carbon is bonded to an electronegative atom, Nitrogen. Denoted as 'b'

NMR analysis of our compounds was thus able to help us understand the structure, identity and the chemical environment of the molecules.

As a whole we were able to synthesize our target molecule.

4.2 Biological Evaluation of the compound (Compound 1)

METHODOLOGY

SH-SY5Y neuroblastoma cells were maintained in standard culture conditions, with regular thawing, splitting and freezing as required. The steps are as follows:

- THAWING OF CELLS

Cryovials were taken out from liquid nitrogen and rapidly thawed at 37°C water bath by gently agitating until only a small ice crystal remained. Then the vial is sterilised with 70% ethanol and transferred to a sterile environment.The solutions that contained the cells were seeded in a culture dish. The cells were maintained overnight in an incubator set to 37°C with 5% CO_2. The next day, the medium was replaced with new media to eliminate residual DMSO to avoid the DMSO mediated cell toxicity.

- SPLITTING OF CELLS

Cells were subcultured when they reached approximately 90–95% confluency. The culture medium was first removed, and the cell monolayer was gently rinsed once with 1X PBS (pH 7.4) to eliminate any residual serum that could inhibit trypsin activity. To detach the cells, 500 µL to 1 mL of 0.25% Trypsin-EDTA solution was put into each culture dish.

The cultures were then incubated at 37°C with 5% CO_2 for about 5 minutes to facilitate cell detachment. After confirming cell detachment under a microscope, an equal volume of complete growth medium was added to neutralize the trypsin. The cell suspension was collected and centrifuged at 1000 × g for 5 minutes. We discarded the supernatant and the cell pellet was resuspended in 1 mL of DMEM, this is supplemented with 10% FBS. For cell counting, 10 µL of the cell suspension was mixed with 50 µL of trypan blue, resulting in a 6X dilution. Then, 10 µL of this mixture was loaded onto a hemocytometer, and viable cells were counted. Finally, cells were seeded at a density of 1×10^5 cells/mL in fresh culture medium and incubated at 37°C with 5% CO_2 for further growth.

- FREEZING OF CELLS

For cryopreservation, cells at 85–95% confluency were harvested as above, resuspended in freezing medium (DMEM with 10% FBS and 10% DMSO), aliquoted into cryovials, and gradually frozen before storage in liquid nitrogen. Cells were seeded at a density of 50,000 cells per well in 6-well plates, while for 96-well plates, seeding densities ranged from 10,000 to 20,000 cells per well, depending on the experimental requirementsUpon reaching 50-60% confluency, neuronal cells in 6-well plates were divided into the following groups, all maintained in DMEM without FBS:

- Naive control (media change only)
- Vehicle control (0.1% DMSO)
- 1 µM of Compound 1
- 10 µM of Compound 1

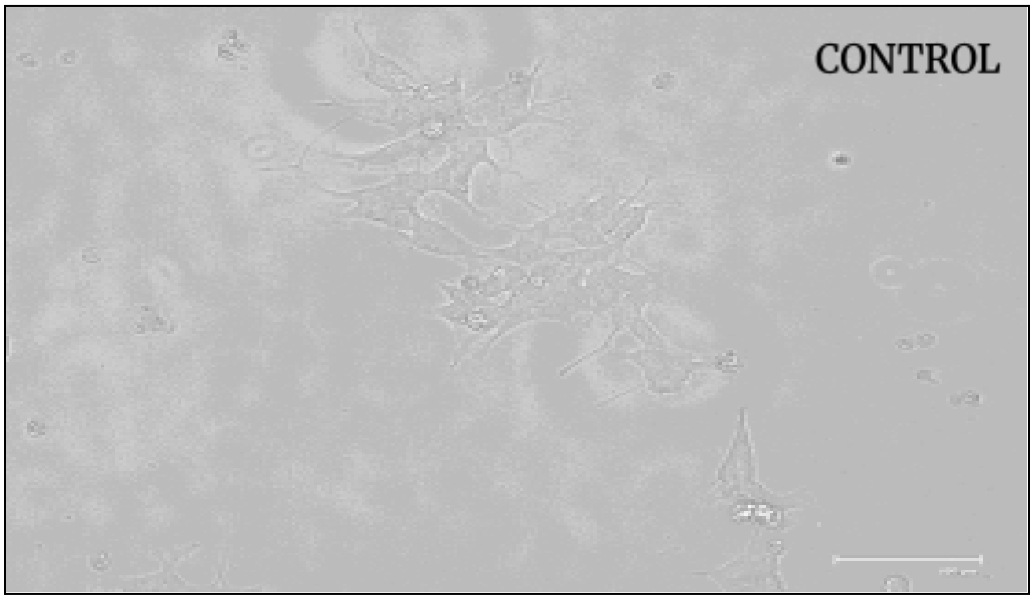
Treatments were applied for 48 hours. Prior to RNA isolation, cell morphology was documented using microscopy.

Subsequently, the media was removed, and 1 ml of Trizol reagent was added to each well for RNA extraction.

MTT assay was conducted for assessing cell viability. Absorbance was measured at 570 nm and subsequent data analysis indicated that the compound exhibited no cytotoxic effects on the cells.

4.2.1 RESULTS OF BIOLOGICAL EVALUATION

BEFORE TREATMENT WITH COMPOUND 1



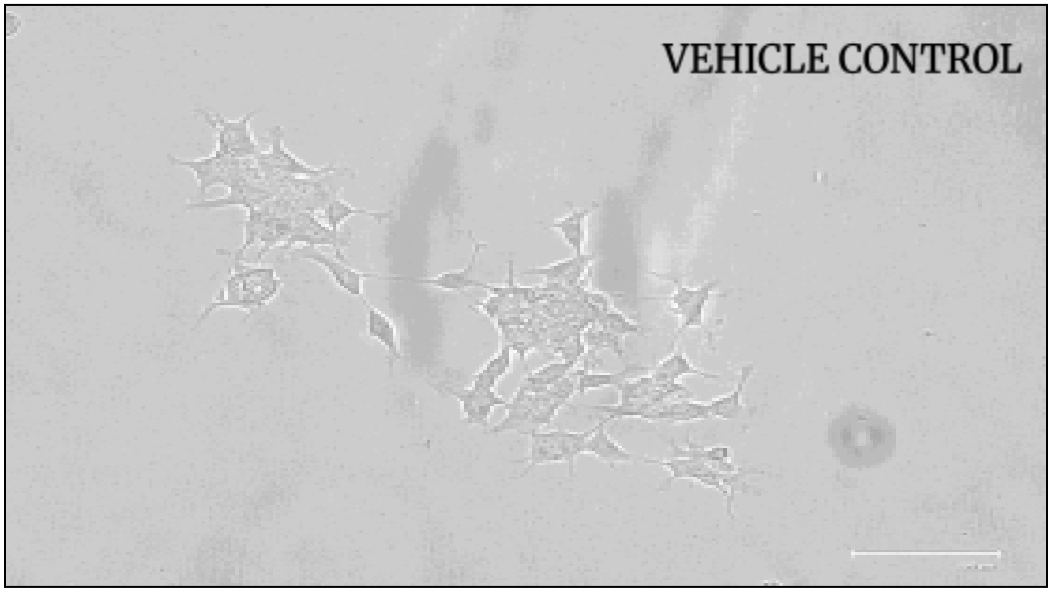
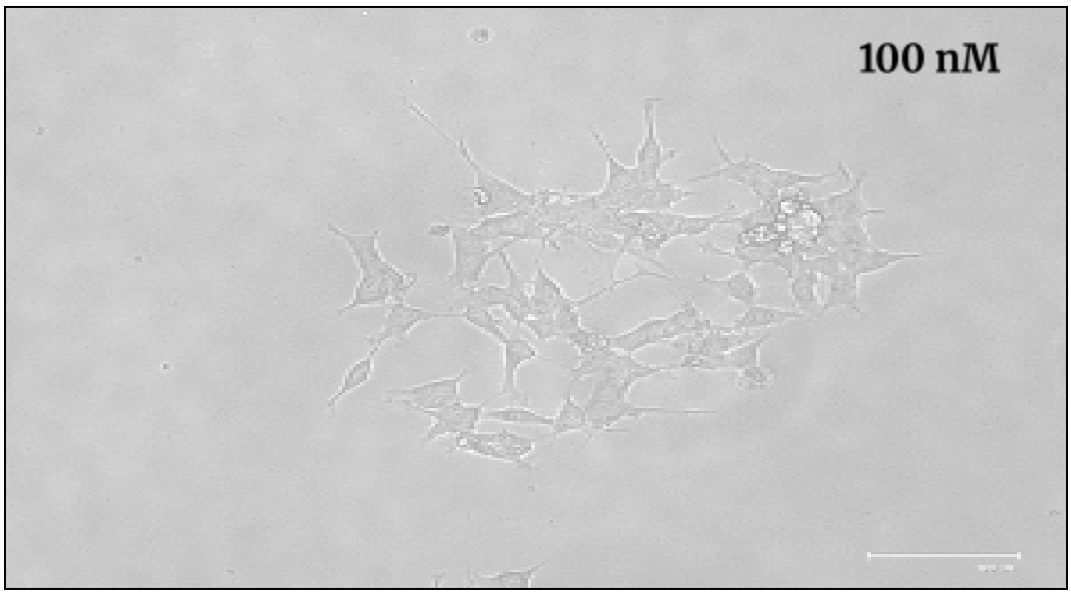


Figure 10 & 11: Representative Phase Contrast Microscopic images of SH-SY5Y neuroblastoma cells before treatment. Control (Figure 10): Cells were subjected to media change only, no DMSO. They are exhibiting normal morphology. Vehicle Control (Image 11): Cells treated with 0.1% DMSO in DMEM without FBS. Cells did not show any significant alterations as compared to Control.

AFTER TREATMENT WITH COMPOUND 1



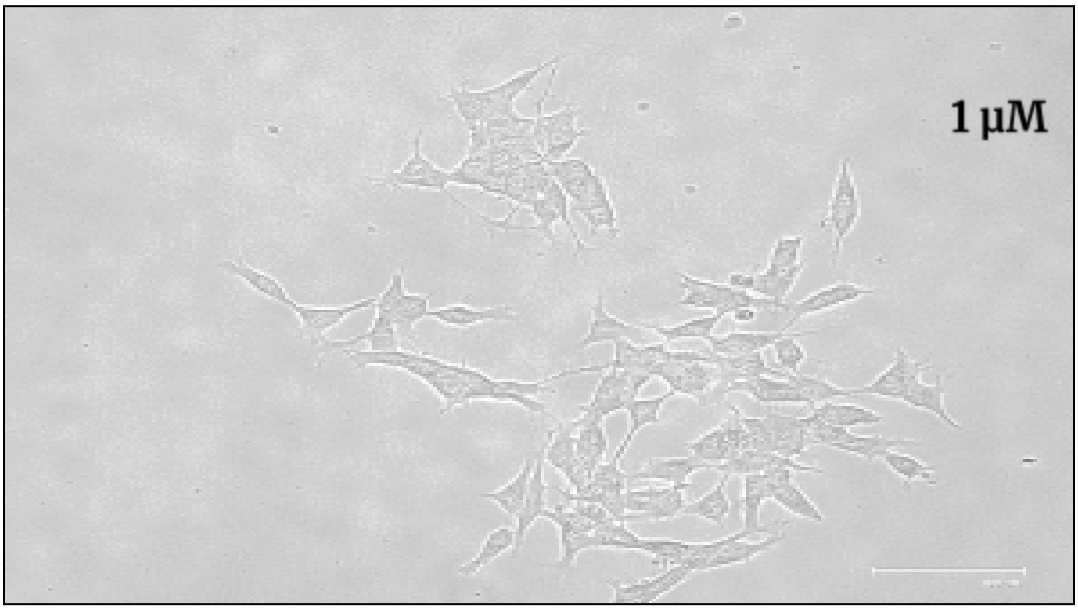


Figure 12 & 13: We tested our compound on SH-SY5Y neuronal cells at concentrations of 100nM (Figure 12) and 1µM (Figure 13). At both concentrations, the cells exhibited pronounced neuronal networking, with extensive neurite outgrowth and interconnected processes, closely resembling the morphology and network formation typically induced by serotonin.

CONCLUSION

In this study, two novel pharmacophores (compound 1 and 2) were successfully synthesized using green chemistry principles and innovative synthetic methods, this highlights our awareness towards the impact of hazardous chemicals and wastes produced during conventional synthesis methods on our environment. We explored several reactions that could possibly give us a compound with neurotransmitter-like abilities. The structure and purity were thoroughly analysed by using analytical methods like ^{1}H-NMR, ^{13}C-NMR. Furthermore the biological testing of our compounds showed encouraging outcomes. Upon treating neural cells with our synthesized compound we found significant proliferation and growth, their well connected synaptic networks indicated communication among the cells, we could say that it closely mimicked Serotonin. Finally, cytotoxicity assays confirmed that our compound showed no cytotoxicity, this proves its biocompatibility and safety.

As this is an ongoing research project, further extensive experiments are crucial to thoroughly investigate the working and action of our compound. Therefore, the comprehensive dataset will remain undisclosed until a more complete understanding of Compound 1′s full biological profile is achieved.

Finally, these findings highlight the potential of these green-synthesized compounds to promote healthy neuronal development and function, paving the way for future neurobiological and therapeutic applications.

REFERENCES

[1] Teleanu, R. I., Niculescu, A. G., Roza, E., Vladâcenco, O., Grumezescu, A. M., & Teleanu, D. M. (2022). Neurotransmitters—key factors in neurological and neurodegenerative disorders of the central nervous system. International journal of molecular sciences, 23(11), 5954

[2] Caire, M. J., Reddy, V., & Varacallo, M. (2018). Physiology, synapse.

[3] Axelrod J. (1974). Neurotransmitters. Scientific American, 230(6), 59–71.

[4] Lovinger, D. M. (2008). Communication networks in the brain: neurons, receptors, neurotransmitters, and alcohol. Alcohol Research & Health, 31(3), 196.

[5] Nguyen, L. R. (2001). Neurotransmitters as early signals for central nervous system development. *Cell Tissue Res 305* , 187–202.

[6] Banskota, S., Ghia, J. E., & Khan, W. I. (2019). Serotonin in the gut: Blessing or a curse. *Biochimie*, *161*, 56-64.

[7] Pourhamzeh, M., Moravej, F. G., Arabi, M., Shahriari, E., Mehrabi, S., Ward, R., ... & Joghataei, M. T. (2022). The roles of serotonin in neuropsychiatric disorders. *Cellular and molecular neurobiology*, *42*(6), 1671-1692.

[8] Ciranna, Á. (2006). Serotonin as a modulator of glutamate-and GABA-mediated neurotransmission: implications in physiological functions and in pathology. *Current neuropharmacology*, *4*(2), 101-114.

[9] Wu, D., Chen, Q., Chen, X., Han, F., Chen, Z., & Wang, Y. (2023). The blood–brain barrier: Structure, regulation and drug delivery. *Signal transduction and targeted therapy*, *8*(1), 217.

[10] Upadhyay, R. K. (2014). Drug delivery systems, CNS protection, and the blood brain barrier. *BioMed research international*, *2014*(1), 869269.

[11] Ciurea, A. V., Mohan, A. G., Covache-Busuioc, R. A., Costin, H. P., Glavan, L. A., Corlatescu, A. D., & Saceleanu, V. M. (2023). Unraveling molecular and genetic insights into neurodegenerative diseases: Advances in understanding Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis. *International journal of molecular sciences*, *24*(13), 10809.

[12] Dong, X. (2018). Current strategies for brain drug delivery. *Theranostics*, *8*(6), 1481.
[13] Anastas, P. T., & Beach, E. S. (2007). Green chemistry: the emergence of a transformative framework. *Green Chemistry Letters and Reviews*, *1*(1), 9-24.

[14] Kar, S., Sanderson, H., Roy, K., Benfenati, E., & Leszczynski, J. (2021). Green chemistry in the synthesis of pharmaceuticals. *Chemical Reviews*, *122*(3), 3637-3710.

[15] Hamid, H. A., Ramli, A. N., & Yusoff, M. M. (2017). Indole Alkaloids from Plants as Potential Leads for Antidepressant Drugs: A Mini Review. *Frontiers in pharmacology*, *8*, 96. https://doi.org/10.3389/fphar.2017.00096

[16] Silverman, R. B., Lawton, G. R., Ralay Ranaivo, H., Chico, L. K., Seo, J., & Watterson, D. M. (2009). Effect of potential amine prodrugs of selective neuronal nitric oxide synthase inhibitors on blood-brain barrier penetration. *Bioorganic & medicinal chemistry*, *17*(21), 7593–7605. https://doi.org/10.1016/j.bmc.2009.08.065

[17] Xiang, Y., Xiang, M., Mao, Y., Huang, L., He, Q., & Dong, Y. (2025). Insights into structure-antioxidant activity relationships of polyphenol-phospholipid complexes: The effect of hydrogen bonds formed by phenolic hydroxyl groups. *Food Chemistry*, 144471.

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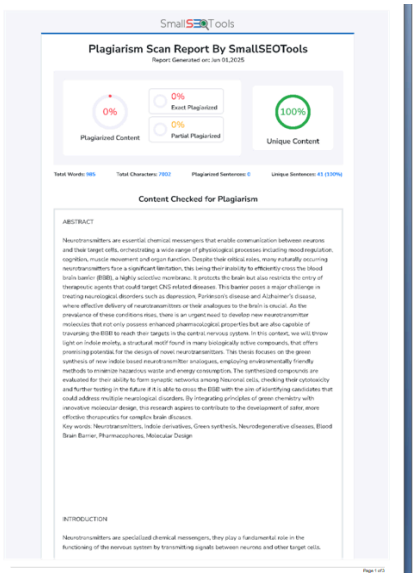
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ABSTRACT

Neurotransmitters are essential chemical messengers that enable communication between neurons and their target cells, orchestrating a wide range of physiological processes including neuroregulation, cognition, muscle movement and organ function. Despite their critical roles, many naturally occurring neurotransmitters face a significant limitation, this being their inability to efficiently cross the blood brain barrier (BBB), a highly selective membrane. It protects the brain but also restricts the entry of therapeutic agents that could target CNS related diseases. This barrier poses a major challenge in treating neurological disorders such as depression, Parkinson's disease and Alzheimer's disease, where effective delivery of neurotransmitters or their analogues to the brain is crucial. As the prevalence of these conditions rises, there is an urgent need to develop new neurotransmitter molecules that not only possess enhanced pharmacological properties but are also capable of traversing the BBB to reach their targets in the central nervous system. In this context, we will throw light on indole moiety, a structural motif found in many biologically active compounds, that offers promising potentials for the design of novel neurotransmitters. This thesis focuses on the green synthesis of new indole based neurotransmitter analogues, employing environmentally friendly methods to minimize hazardous waste and energy consumption. The synthesized compounds are evaluated for their ability to form synaptic networks among Neuronal cells, checking their cytotoxicity and further testing in the future if it is able to cross the BBB with the aim of identifying candidates that could address multiple neurological disorders. By integrating principles of green chemistry with innovative molecular design, this research aspires to contribute to the development of safer, more effective therapeutics for complex brain diseases.

Key words: Neurotransmitters, Indole derivatives, Green synthesis, Neurodegenerative diseases, Blood Brain Barrier, Pharmacophores, Molecular Design

INTRODUCTION

Neurotransmitters are specialized chemical messengers, they play a fundamental role in the functioning of the nervous system by transmitting signals between neurons and other target cells.

They are really important for regulating several psychological and physiological processes, this includes our mood, movement, heartbeat and blood pressure, hormone regulation, memory, digestion etc.[1] Synapses are the specialized points where neurons communicate. Each synapse features two main parts: a postsynaptic and a presynaptic terminal. The release of NTs from the presynaptic neuron into the synaptic cleft is initiated by an electrical impulse. These NTs then bind to postsynaptic receptors, either stimulating or inhibiting subsequent signaling. [2] Based on their effects, neurotransmitters are broadly classified as excitatory, inhibitory, or modulatory. Major classes include amino acids (e.g. glutamate, GABA), monoamines (e.g. dopamine, norepinephrine, epinephrine, serotonin), peptides (e.g. endorphins), purines, and others.[3][4] Neurotransmitters hence are in control of several actions that is necessary for our survival, but when our body faces an imbalance or dysfunctions in their signaling, presence or action, it implies a variety of neurological and psychiatric disorders. [5] Now among all these we shall throw light on Serotonin (5-hydroxytryptamine or 5-HT), a monoamine neurotransmitter that also acts as a hormone. Studies have shown how 5-HT has a strong influence on both peripheral and physiological activities.[6] In our human body, almost 95% of 5-HT is found in the gut (enterochromaffin cells) and the remaining 5% in the brain. [6] It is able to regulate our mood, digestion, sleep etc. Low levels can cause conditions such as depression, anxiety, migraine, and Parkinson's disease.[7] Not only this but it can also modulate other neurotransmitters' activity, this includes dopamine, glutamate and GABA. [8]

Serotonin hence is of high importance but these comes a huge problem i.e the Blood Brain Barrier (BBB). The brain is protected by the blood-brain barrier (BBB), a specialized membrane that allows only certain substances to pass through due to its highly selective and semi permeable nature. It restricts the entry of toxins, pathogens and even several therapeutic agents, this includes most of the naturally occurring neurotransmitters and their analogues. [9] Because of this limitation numerous CNS related diseases and disorders are not getting effective management and treatment since when these neurotransmitters are administered exogenously, they fail to reach the target within the brain. [10] As per recent studies it was found that neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease are characterized by progressive loss of neuronal structure and function, leading to weakening cognitive and motor deficits, they are also one of the main causes of disability and death worldwide. [11] Current therapeutic strategies are limited by poor BBB permeability, suboptimal efficacy, and undesirable side effects, underscoring the necessity for innovative approaches in drug design and delivery. [12] To tackle this problem it is the need of the hour to design and synthesize neurotransmitter analogues that not only enhances the properties of NTs but can also cross the BSB. This could revolutionize medicine and introduce effective ways to treat patients in need. In recent years, green chemistry has emerged as a transformative approach in pharmaceutical synthesis, emphasizing the use of environmentally friendly solvents, renewable resources and energy efficient processes. The application of green chemistry principles to the synthesis of indole-based neurotransmitter analogues offers the dual advantage of reducing environmental impact and generating safer, more sustainable small molecules that can mimic a neurotransmitter. One of the common things among several biologically active molecules is that they all have an indole ring, for example- 5-HT. By using green synthetic methodologies, it is possible to design and produce novel indole-based neurotransmitters with properties tailored for enhanced BSB penetration and therapeutic efficacy. [13], [14]

This thesis aims to address the urgent need for new neurotransmitter analogues capable of overcoming the limitations imposed by the BBB. The research focuses on the green synthesis of indole-based neurotransmitters, followed by their evaluation in biological systems to assess cytotoxicity, how well it helps the neuronal cells proliferate and communicate and BBB permeability and efficacy against neurodegenerative disease models for future studies. By integrating advances in green chemistry with neuropharmacological innovation, this work aspires to contribute to the development of next generation therapeutics for CNS disorders.

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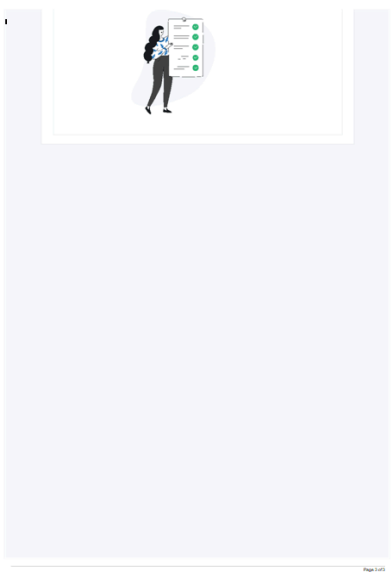
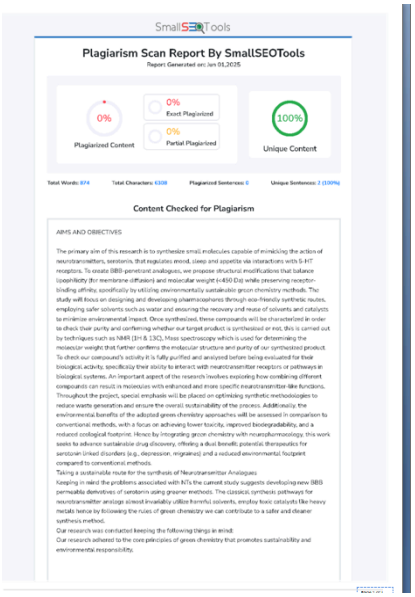
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AIMS AND OBJECTIVES

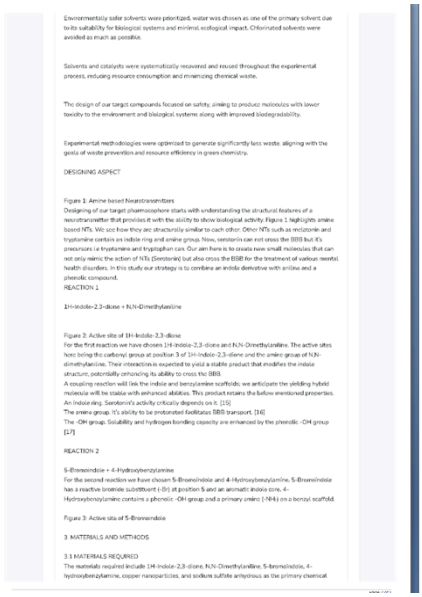
The primary aim of this research is to synthesize small molecules capable of mimicking the action of neurotransmitters, serotonin, that regulates mood, sleep and appetite via interactions with 5-HT receptors. To create BBB-penetrant analogues, we propose structural modifications that balance Lipophilicity (for membrane diffusion) and molecular weight (<450 Da) while preserving receptor-binding affinity, specifically by utilizing environmentally sustainable green chemistry methods. The study will focus on designing and developing pharmacophores through eco-friendly synthetic routes, employing safer solvents such as water and ensuring the recovery and reuse of solvents and catalysts to minimize environmental impact. Once synthesized, these compounds will be characterized in order to check their purity and confirming whether our target product is synthesized or not, this is carried out by techniques such as NMR (1H & 13C), Mass spectroscopy which is used for determining the molecular weight that further confirms the molecular structure and purity of our synthesized product. To check our compound's activity it is fully purified and analyzed before being evaluated for their biological activity, specifically their ability to interact with neurotransmitter receptors or pathways in biological systems. An important aspect of the research involves exploring how combining different compounds can result in molecules with enhanced and more specific neurotransmitter-like functions. Throughout the project, special emphasis will be placed on optimizing synthetic methodologies to reduce waste generation and ensure the overall sustainability of the process. Additionally, the environmental benefits of the adopted green chemistry approaches will be assessed in comparison to conventional methods, with a focus on achieving lower toxicity, improved biodegradability, and a reduced ecological footprint. Hence, by integrating green chemistry with neuropharmacology, this work seeks to advance sustainable drug discovery, offering a dual benefit, potential therapeutics for serotonin linked disorders (e.g., depression, migraines) and a reduced environmental footprint compared to conventional methods.

Taking a sustainable route for the synthesis of Neurotransmitter Analogues

Keeping in mind the problems associated with NTs the current study suggests developing new BBB permeable derivatives of serotonin using greener methods. The classical synthesis pathways for neurotransmitter analogs stressed invariably utilize harmful solvents, smelley toxic catalysts like heavy metals hence by following the rules of green chemistry we can contribute to a safer and cleaner synthesis method.

Our research was conducted keeping the following things in mind:

Our research adhered to the core principles of green chemistry that promotes sustainability and environmental responsibility.


Environmentally safer solvents were prioritized, water was chosen as one of the primary solvent due to its suitability for biological systems and minimal ecological impact. Chlorinated solvents were avoided as much as possible.

Solvents and catalysts were systematically recovered and reused throughout the experimental process, reducing resource consumption and minimizing chemical waste.

The design of our target compounds focused on safety, aiming to produce molecules with lower toxicity to the environment and biological systems along with improved biodegradability.

Experimental methodologies were optimized to generate significantly less waste, aligning with the goals of waste prevention and resource efficiency in green chemistry.

DESIGNING ASPECT

Figure 1: Amine based Neurotransmitters

Designing of our target pharmacophore starts with understanding the structural features of a neurotransmitter that provides it with the ability to show biological activity. Figure 1 highlights amine based NTs. We see how they are structurally similar to each other. Other NTs such as melatonin and tryptamine contain an indole ring and amine group. Now, serotonin can not cross the BBB but it's precursors i.e tryptamine and tryptophan can. Our aim here is to create new small molecules that can not only mimic the action of NTs (Serotonin) but also cross the BSB for the treatment of various mental health disorders. In this study our strategy is to combine an indole derivative with aniline and a phenolic compound.

REACTION 1

1H-Indole-2,3-dione + N,N-Dimethylaniline

Figure 2: Active site of 1H-Indole-2,3-dione

For the first reaction we have chosen 1H-Indole-2,3-dione and N,N-Dimethylaniline. The active sites here being the carbonyl group at position 3 of 1H-Indole-2,3-dione and the amine group of N,N-dimethylaniline. Their interaction is expected to yield a stable product that modifies the indole structure, potentially enhancing its ability to cross the BBB.

A coupling reaction will link the indole and benzylamine scaffolds; we anticipate the yielding hybrid molecule will be stable with enhanced abilities. This product retains the below mentioned properties.

An indole ring: Serotonin's activity critically depends on it. [15]

The amine group. It's ability to be protonated facilitates BBB transport. [16]

The -OH group. Solubility and hydrogen bonding capacity are enhanced by the phenolic -OH group [17]

REACTION 2

5-Bromoindole + 4-Hydroxybenzylamine

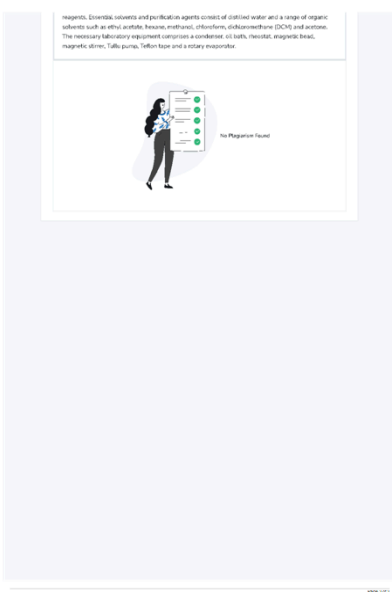
For the second reaction we have chosen 5-Bromoindole and 4-Hydroxybenzylamine. 5-Bromoindole has a reactive bromide substituent (-Br) at position 5 and an aromatic indole core. 4-Hydroxybenzylamine contains a phenolic -OH group and a primary amine (-NH2) on a benzyl scaffold.

Figure 3: Active site of 5-Bromoindole

3. MATERIALS AND METHODS

3.1 MATERIALS REQUIRED

The materials required include 1H-Indole-2,3-dione, N,N-Dimethylaniline, 5-bromoindole, 4-hydroxybenzylamine, copper nanoparticles, and sodium sulfate anhydrous as the primary chemical


reagents. Essential solvents and purification agents consist of distilled water and a range of organic solvents such as ethyl acetate, hexane, methanol, chloroform, dichloromethane (DCM) and acetone. The necessary laboratory equipment comprises a condenser, oil baths, rheostat, magnetic bead, magnetic stirrer, Tullu pump, Teflon tape and a rotary evaporator.

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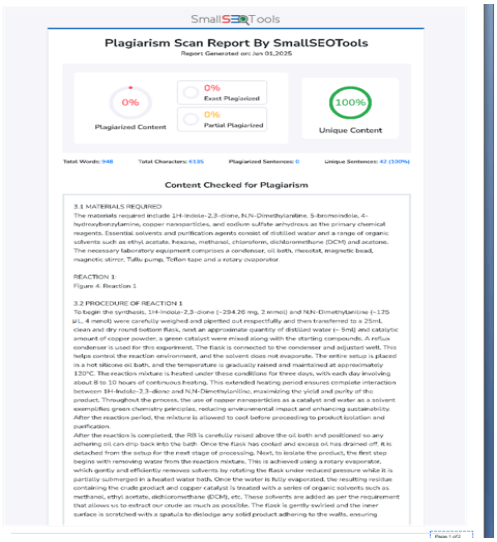
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3.1 MATERIALS REQUIRED

The materials required include 1H-Indole-2,3-dione, N,N-Dimethylaniline, 5-Bromoindole, 4-hydroxybenzylamine, copper nanoparticles, and sodium sulfate anhydrous as the primary chemical reagents. Essential solvents and purification agents consist of distilled water and a range of organic solvents such as ethyl acetate, hexane, methanol, chloroform, dichloromethane (DCM) and acetone. The necessary laboratory equipment comprises a condensor, oil bath, rheostat, magnetic bead, magnetic stirrer, Teflo pump, Teflon tape and a rotary evaporator.

REACTION 1:

Figure 4: Reaction 1

3.2 PROCEDURE OF REACTION 1

To begin the synthesis, 1H-Indole-2,3-dione (~294.26 mg, 2 mmol) and N,N-Dimethylaniline (~1.25 µL, 4 mmol) were carefully weighed and pipetted out respectfully and then transferred to a 25mL clean and dry round bottom flask, next an approximate quantity of distilled water (~ 5mL) and catalytic amount of copper powder, a green catalyst were mixed along with the starting compounds. A reflux condenser is used for this experiment. The flask is connected to the condenser and adjusted well. This helps control the reaction environment, and the solvent does not evaporate. The entire setup is placed in a hot silicone oil bath, and the temperature is gradually raised and maintained at approximately 120°C. The reaction mixture is heated under these conditions for three days, with each day involving about 8 to 10 hours of continuous heating. This extended heating period ensures complete interaction between 1H-Indole-2,3-dione and N,N-Dimethylaniline, maximizing the yield and purity of the product. Throughout the process, the use of copper nanoparticles as a catalyst and water as a solvent exemplifies green chemistry principles, reducing environmental impact and enhancing sustainability.

After the reaction period, the mixture is allowed to cool before proceeding to product isolation and purification.

After the reaction is completed, the RB is carefully raised above the oil bath and positioned so any adhering oil can drip back into the bath. Once the flask has cooled and excess oil has drained off, it is detached from the setup for the next stage of processing. Next, to isolate the product, the first step begins with removing water from the reaction mixture. This is achieved using a rotary evaporator, which gently and efficiently removes solvents by rotating the flask under reduced pressure while it is partially submerged in a heated water bath. Once the water is fully evaporated, the resulting residue containing the crude product and copper catalyst is treated with a series of organic solvents such as methanol, ethyl acetate, dichloromethane (DCM), etc. These solvents are added as per the requirement that allows us to extract our crude as much as possible. The flask is gently swirled and the inner surface is scratched with a spatula to dislodge any solid product adhering to the walls, ensuring

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maximum recovery.

Next, the mixture sits undisturbed, allowing the denser copper nanoparticles and any insoluble material to settle. The supernatant (which holds the crude product dissolved in organic solvents) is carefully decanted into a clean china dish. Silica gel powder is then introduced and mixed well. This silica gel functions as an adsorbent, effectively capturing the product to aid in its later purification. The mixture of silica and product is left to air dry in a china dish, allowing the solvent to evaporate. this leaves behind the crude product adsorbed onto silica. This process is repeated multiple times: fresh solvent is added to the original flask, more product is extracted and transferred to the china dish, and additional silica gel is used as needed. This iterative extraction ensures that as much product as possible is recovered from the reaction mixture. This systematic approach—combining careful solvent removal, efficient extraction, sedimentation, and adsorption—maximizes product yield and purity, setting the stage for subsequent purification steps such as column chromatography.

3.3 PURIFICATION OF CRUDE PRODUCT

The purification of the crude product is accomplished using column chromatography, a technique that is used to separate compounds based on their differential affinities for a stationary phase (silica gel) and a mobile phase (organic solvents).

Column Preparation

A clean, dry glass chromatography column was taken and prepared by cotton plugging it at the base. For the stationary phase silica gel was chosen, using our mobile phase solvent Le Hexane we prepared a slurry that was evenly wetted and free from air bubbles. The slurry was hence poured and the solvent was allowed to drain until it just reached the surface of the silica, making sure the column never dries. The length was decided upon keeping in mind that we wanted a longer column for better separation.

Sample Loading

Our crude was in dry state since we already adsorbed it in a small amount of silica gel. For loading, the powder was transferred onto the top of the silica bed, making sure that it's even. After this it was cotton plugged just above the sample bed and a small amount of hexane was poured to settle the sample and begin the elution process.

Elution and Fraction Collection

Once the column was prepared we began elution with pure hexane as the mobile phase, gradually increasing the polarity by introducing Ethyl acetate in incremental concentrations. We started with 2% Ethyl acetate in Hexane, creating this gradual gradient allows efficient separation of compounds based on their polarity. At lower polarity non polar compounds elute first, polar compounds on the other hand retain for longer, only eluting out when the polarity is increased. To isolate the desired compound after column chromatography, we evaporate the solvents from the collected fractions using a rotary evaporator. This process yields a solid residue within the round-bottom flask, which we must handle with care to prevent any loss or contamination. An added benefit is that the solvent collected by the evaporator can be reused for the columns, promoting efficiency.

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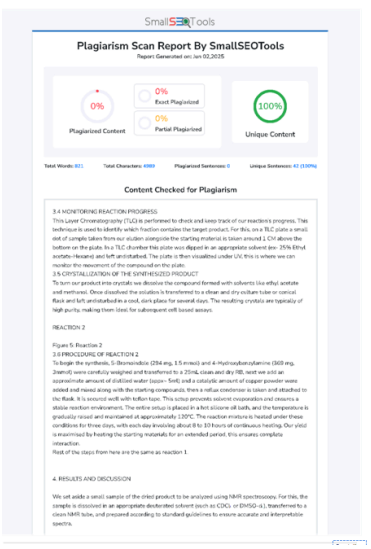
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3.4 MONITORING REACTION PROGRESS

This Layer Chromatography (TLC) is performed to check and keep track of our reaction's progress. This technique is used to identify which fraction contains the target product. For this, on a TLC plate a small dot of sample taken from our eluden alongside the starting material is taken around 1 CM above the bottom on the plate. In a TLC chamber this plate was dipped in an appropriate solvent (ex- 25% Ethyl acetate-Hexane) and left undisturbed. The plate is then visualized under UV, this is where we can monitor the movement of the compound on the plate.

3.5 CRYSTALLIZATION OF THE SYNTHESIZED PRODUCT

To turn our product into crystals we dissolve the compound formed with solvents like ethyl acetate and methanol. Once dissolved the solution is transferred to a clean and dry culture tube or conical flask and left undisturbed in a cool, dark place for several days. The resulting crystals are typically of high purity, making them ideal for subsequent cell based assays.

REACTION 2

Figure 5: Reaction 2

3.6 PROCEDURE OF REACTION 2

To begin the synthesis, 5-Bromoindole (294 mg, 1.5 mmol) and 4-Hydroxybenzylamine (369 mg, 2mmol) were carefully weighed and transferred to a 25mL clean and dry RB, next we add an approximate amount of distilled water (appx- 5ml) and a catalytic amount of copper powder were added and mixed along with the starting compounds, then a reflux condenser is taken and attached to the flask. It is secured well with teflon tape. This setup prevents solvent evaporation and ensures a stable reaction environment. The entire setup is placed in a hot silicone oil bath, and the temperature is gradually raised and maintained at approximately 120°C. The reaction mixture is heated under these conditions for three days, with each day involving about 8 to 10 hours of continuous heating. Our yield is maximised by heating the starting materials for an extended period, this ensures complete interaction.

Rest of the steps from here are the same as reaction 1.

4. RESULTS AND DISCUSSION

We set aside a small sample of the dried product to be analyzed using NMR spectroscopy. For this, the sample is dissolved in an appropriate deuterated solvent (such as CDCl3 or DMSO-d6), transferred to a clean NMR tube, and prepared according to standard guidelines to ensure accurate and interpretable spectra.

4.1 NMR ANALYSIS

COMPOUND 1

4.1.1 Characterization of Compound 1 using 1H-NMR (600MHz)

The above 1H NMR spectrum indicates that:

On the graph, b represents H from the N-CH3 groups. In total there are 12 H-protons. Peaks appeared at δ2.51 ppm. This is the representation of Aliphatic protons.

A singlet at δ 10.51 ppm represents 1H associated with -NH. This represents the Acidic proton.

The region between δ6.63 to δ 7.21 ppm the Aromatic protons.

4.1.2 Characterization of Compound 1 using 13C-NMR. (150MHz)

The above 13C NMR spectrum indicates that:

At δ 40.06 ppm the peak represents a methyl group attached to a Nitrogen atom (N-CH3), denoted as b

From δ 112.59 to δ 149.69 ppm the peaks represent Aromatic carbons.

The singlet at δ 179.77 ppm, represents a carbonyl carbon (C=O), denoted as a

Here 'c' is a quaternary carbon.

COMPOUND 2

4.1.3 Characterization of Compound 2 using 1H-NMR. (600MHz)

The above 1H NMR spectrum indicates that:

A signal at δ 3.89 ppm is representing an Aliphatic proton (-CH2)

Aromatic protons fall between the range of δ 6.77 ppm to δ 8.03 ppm in this graph.,

At δ 5.48 ppm, the peak represents an aromatic proton. It is assigned to a carbon that is attached with an electronegative element like nitrogen.

Characterization of Compound 2 using 13C-NMR. (150MHz)

The above 13C NMR spectrum indicates that:

Between δ 112.8 ppm to δ 139.1 ppm we can observe the Aromatic Carbons present in our compound.

The Aliphatic carbon gives the signal at δ 30.5 ppm, Denoted as 'a'

At the signal observed at δ 158.7 ppm there is an aromatic carbon. This carbon is bonded to an electronegative atom, Nitrogen. Denoted as 'e'

NMR analysis of our compounds was thus able to help us understand the structure, identity and the chemical environment of the molecules. As a whole we could synthesize our target molecule.

4.2 Biological Evaluation of the compound

(Compound 1)

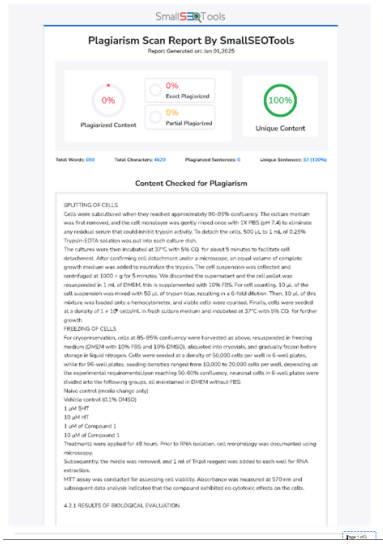
METHODOLOGY

SH-SY5Y neuroblastoma cells were maintained in standard culture conditions, with regular thawing, splitting and freezing as required. The steps are as follows:

THAWING OF CELLS

Cryovials were taken out from liquid nitrogen and rapidly thawed at 37°C water bath by gently agitating until only a small ice crystal remained. Then the vial is sterilised with 70% ethanol and

transferred to a sterile environment The solutions that contained the cells were seeded in a culture dish. The cells were maintained overnight in an incubator set to 37°C with 5% CO2. The next day, the medium was replaced with new media to eliminate residual DMSO to avoid the DMSO-mediated cell toxicity.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SPLITTING OF CELLS

Cells were subcultured when they reached approximately 90-95% confluency. The culture medium was first removed, and the cell monolayer was gently rinsed once with 1X PBS (pH 7.4) to eliminate any residual serum that could inhibit trypsin activity. To detach the cells, 500 µL to 1 mL of 0.25% Trypsin-EDTA solution was put into each culture dish.

The cultures were then incubated at 37°C with 5% CO2 for about 5 minutes to facilitate cell detachment. After confirming cell detachment under a microscope, an equal volume of complete growth medium was added to neutralize the trypsin. The cell suspension was collected and centrifuged at 1000 × g for 5 minutes. We discarded the supernatant and the cell pellet was resuspended in 1 mL of EMEM, this is supplemented with 10% FBS. For cell counting, 10 µL of the cell suspension was mixed with 50 µL of trypan blue, resulting in a 6-fold dilution. Then, 10 µL of this mixture was loaded onto a hemocytometer, and viable cells were counted. Finally, cells were seeded at a density of 1 × 10⁶ cells/mL in fresh culture medium and incubated at 37°C with 5% CO2 for further growth.

FREEZING OF CELLS

For cryopreservation, cells at 85-95% confluency were harvested as above, resuspended in freezing medium (DMEM with 10% FBS and 10% DMSO), aliquoted into cryovials, and gradually frozen before storage in liquid nitrogen. Cells were seeded at a density of 50,000 cells per well in 6-well plates, while for 96-well plates, seeding densities ranged from 10,000 to 20,000 cells per well, depending on the experimental requirements.Upon reaching 50-60% confluency, neuronal cells in 6-well plates were divided into the following groups, all maintained in DMEM without FBS:

Naive control (media change only)

Vehicle control (0.1% DMSO)

1 µM SHT

10 µM HT

1 µM of Compound 1

10 µM of Compound 1

Treatments were applied for 48 hours. Prior to RNA isolation, cell morphology was documented using microscopy.

Subsequently, the media was removed, and 1 ml of Trizol reagent was added to each well for RNA extraction.

MTT assay was conducted for assessing cell viability. Absorbance was measured at 570 nm and subsequent data analysis indicated that the compound exhibited no cytotoxic effects on the cells.

4.2.1 RESULTS OF BIOLOGICAL EVALUATION

BEFORE TREATMENT WITH COMPOUND 1

Figure 10 & 11: Representative Phase Contrast Microscopic images of SH-SY5Y neuroblastoma cells before treatment. Control (Figure 10): Cells were subjected to media change only, no DMSO. They are exhibiting normal morphology. Vehicle Control (Image 11): Cells treated with 0.1% DMSO in EMEM without FBS. Cells did not show any significant alterations as compared to Control.

AFTER TREATMENT WITH COMPOUND 1

Figure 12 & 13: We tested our compound on SH-SY5Y neuronal cells at concentrations of 100nM (Figure 12) and 10nM (Figure 13). At both concentrations, the cells exhibited pronounced neuronal networking, with extensive neurite outgrowth and interconnected processes, closely resembling the morphology and network formation typically induced by serotonin.

CONCLUSION

In this study, two novel pharmacophores (compound 1 and 2) were successfully synthesized using green chemistry principles and innovative synthetic methods, this highlights our awareness towards the impact of hazardous chemicals and wastes produced during conventional synthesis methods on our environment. We explored several reactions that could possibly give us a compound with neurotransmitter-like abilities. The structure and purity were thoroughly analysed by using analytical methods like 1H-NMR, 13C-NMR, and mass spectrometry. Furthermore the biological testing of our compounds showed encouraging outcomes. Upon treating neural cells with our synthesized compound we found significant proliferation and growth, their well connected synaptic networks indicated communication among the cells, we could say that it classy mimicked Serotonin. Finally, cytotoxicity assays confirmed that our compound showed no cytotoxicity, this proves its biocompatibility and safety.

As this is an ongoing research project, further extensive experiments are crucial to thoroughly investigate the working and action of our compound. Therefore, the comprehensive dataset will remain undisclosed until a more complete understanding of Compound 1's full biological profile is achieved. Finally, these findings highlight the potential of these green-synthesized compounds to promote

healthy neuronal development and function, paving the way for future neurobiological and therapeutic applications.